

S/N 09/870,407

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Douglas J. Lacount et al.

Examiner: Unknown

Serial No.: 09/870,407

Group Art Unit: Unknown

Filed: May 30, 2001

Docket: 875.030US1

Title: METHOD OF RAPIDLY GENERATING DOUBLE-STRANDED RNA AND  
METHODS OF USE THEREOF

PRELIMINARY AMENDMENT

Commissioner for Patents  
Washington, D.C. 20231

Sir:

In response to the "Notice to File Missing Parts of Nonprovisional Application" mailed June 22, 2001, please amend the above-identified patent application as follows:

In the Specification

Please enter the enclosed SEQUENCE LISTING into the specification.

Please substitute the paragraph in the appendix entitled "Clean Version of the Paragraph Spanning Pages 23-24" for the paragraph spanning pages 23-24 of the specification. Specific amendments to this paragraph are detailed in the following marked-up paragraph:

To generate p2rRNAprom (Fig. 1A), a 292-bp fragment containing the *T. brucei* rRNA promoter was PCR-amplified with primers that added *Xho*I and *Bam*HI sites to the ends and inserted into the *Sal*I and *Bam*HI sites of pHD496 in the opposite orientation to the rRNA promoter already present (Biebinger S, *et al.* (1996) *Nucleic Acids Res* 24:1202-11). Plasmid p2rRNAprom/ $\alpha$ tub was created by inserting a 486-bp PCR fragment of *T. brucei*  $\alpha$ -tub (60 bp of the 5' UTR and 426 bp of coding region) into the *Hind*III and *Bam*HI sites of p2rRNAprom. A second T7 promoter in the opposite orientation to the T7 promoter already present was added to pBluescriptII SK(-) by annealing oligos 5'-CGTAATACGACTCACTATAGGGCAGCT-3' (SEQ ID NO:1) and 5'-GCCCCTATAGTGAGTCGTATTACGAGCT-3' (SEQ ID NO:2) and ligating into the *Sac*I site of pBluescriptII SK(-) to give p2T7 (Fig. 1A).

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Page 2

Dkt 875.030US1

**Remarks**

This Preliminary Amendment and the above-referenced SEQUENCE LISTING are filed to conform the above-referenced application to the requirements of 37 C.F.R. §§ 1.821 - 1.825. In accordance with 37 C.F.R. § 1.821(e), a copy of the above-submitted SEQUENCE LISTING in ASCII computer readable form is also submitted herewith. The contents of the paper version of the SEQUENCE LISTING and the computer readable form being submitted herewith are the same and do not include new matter.

The amendments to pages 23-24 of the specification adding sequence identifiers are made to conform the above-referenced application to the requirements of 37 C.F.R. § 1.821(d).

Respectfully submitted,

DOUGLAS J. LACOUNT ET AL.

By their Representatives,


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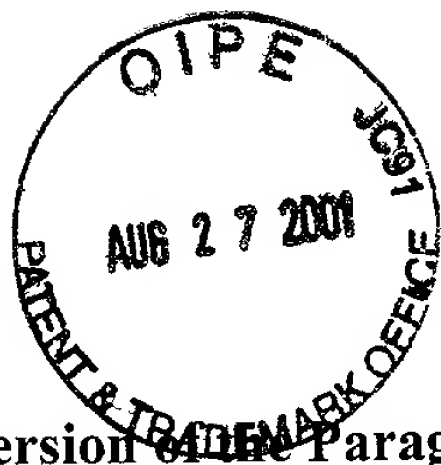
Date 8-22-01 By 

Robert J. Harris  
Reg. No. 37,346  
RJH:CNG:lat

CERTIFICATE UNDER 37 CFR 1.8. The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 22<sup>nd</sup> day of August, 2001

LISA THORIN  
Name

  
Signature



Clean Version of the Paragraph Spanning Pages 23-24

METHOD OF RAPIDLY GENERATING DOUBLE-STRANDED RNA AND METHODS OF  
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To generate p2rRNAprom (Fig. 1A), a 292-bp fragment containing the *T. brucei* rRNA promoter was PCR-amplified with primers that added *Xho*I and *Bam*HI sites to the ends and inserted into the *Sal*I and *Bam*HI sites of pHD496 in the opposite orientation to the rRNA promoter already present (Biebinger S, et al. (1996) *Nucleic Acids Res* 24:1202-11). Plasmid p2rRNAprom/ $\alpha$ tub was created by inserting a 486-bp PCR fragment of *T. brucei*  $\alpha$ -tub (60 bp of the 5' UTR and 426 bp of coding region) into the *Hind*III and *Bam*HI sites of p2rRNAprom. A second T7 promoter in the opposite orientation to the T7 promoter already present was added to pBluescriptII SK(-) by annealing oligos 5'-CGTAATACGACTCACTATAGGGCAGCT-3' (SEQ ID NO:1) and 5'-GCCCCTATAGTGAGTCGTATTACGAGCT-3' (SEQ ID NO:2) and ligating into the *Sac*I site of pBluescriptII SK(-) to give p2T7 (Fig. 1A).